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Influence of Ileo-Caecal Cannulation and Oxytetracycline on Ileo-Caecal and Rectal Coliform Populations in Pigs

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Högberg A, Lindberg JE, Wallgren P: Influence of Ileo-Caecal cannulation and oxytetracycline on Ileo-Caecal and rectal coliform populations in pigs. Acta vet. scand. 2001, 42, 435-440. – The effect of surgery (insertion of an ileo-caecal cannula) and a subsequent parenteral treatment with oxytetracycline on the ileo-caecal and rectal coliform populations in 7 Swedish Yorkshire castrates were studied. Samples were collected during surgery as well as 3, 7, 14 and 20 days post surgery. The diversity of the enteric coliform flora was initially high both in the ileo-caecal ostium and in rectum. No alteration in the diversity of the enteric coliform flora was observed following surgery and treatment with oxytetracycline. As the insertion of ileo-caecal cannulas did not affect the intestinal coliform flora this study gives support to the use of this technique to mirror processes in the small intestine of pigs. Further, the diversity of the enteric coliform flora was unaffected by the parenteral treatment with oxytetracycline.

pig; microbial diversity; coliform, E. coli, ileo-caecal cannula; oxytetracycline.

Introduction

The utilisation of feed is of crucial economical importance when pigs are reared to market weight, and trials aiming to optimise the composition of feed are often performed. One way to examine the nutritional properties of feeds is to compare the content of energy, protein or other compounds in the feed offered with the same parameters in faeces. However, this strategy will only give information about the loss of nutrients during the entire intestinal passage, and some of the nutrients lost could for instance have been used by microorganisms in the large intestine instead of having contributed to the growth of the pig.

The digestive tract is a complex system in the body, responsible for digestion and absorption of nutrients. To fulfil this function the gastro-intestinal canal is divided into compartments that

are responsible for specific processes with respect to the absorption of nutrients, electrolytes and liquids (Mc Donald *et al.* 1995). Consequently, the use of cannulas in strategic spots of the intestine, from which samples could be collected, is of interest when performing studies on nutrient utilisation (Hodgkinson & Moughan 2000). In addition, cannulated pigs may offer a useful model for studies on the interactions between the diet and the enteric microflora.

The microbial flora of the gut is complex and varies between different parts of the intestine (Ewing & Cole 1994). The different floras are characterised by a high number of different species and a high diversity. This high diversity is believed to maintain the enteric flora and to protect from overgrowth of indigenous or exogenous pathological microbes (Kühn *et al.*

1993, *Katouli et al.* 1999). The diversity of the coliform microflora is previously shown to mirror the entire microbial flora, including the anaerobic flora, well in pigs (*Kühn et al.* 1993, 1995, *Katouli et al.* 1995, 1997, *Melin et al.* 1997).

The microbial populations of the intestine may be influenced by altered environmental conditions such as insertion of intestinal cannulas, a phenomenon that not yet has been carefully scrutinised. Further, surgery is often followed by treatment with antibiotics in order to protect the animal from post operation infections. Obviously there is a risk that such treatments, regardless of whether they are given per os or parenterally, influence the enteric microflora. Repeated injections may reflect recurrent stress that theoretically could affect the intestinal flora.

Aiming to scrutinise the relevance of the ileocaecal cannulation technique in nutritional studies we wanted to monitor a possible influence of the insertion of such cannulas on the enteric flora. Further, as postoperative treatments with antibiotics commonly are used to prevent infections, the influence of an antimicrobial treatment on the enteric microflora was studied. Oxytetracycline was used since it is known to bind to divalent ions (*Farrington et al.* 1991, *Carson & Breslyn* 1996) and therefore presumably affect the enteric flora to a lesser extent than other antimicrobials. In this context, also the relative widespread resistance to tetracyclines among coliforms (*Melin et al.* 2000) might contribute to a minor influence on that flora. In the present study a long acting oxytetracycline was given parenterally at one occasion, aiming to stress the pigs as little as possible.

Materials and methods

Animals

Seven Swedish Yorkshire castrates from a con-

ventional herd free from diseases according to the A-list of the International Office of Epizootics, and from Aujeszky's disease, atrophic rhinitis, *Brachyspira* spp, transmissible gastroenteritis, porcine epidemic diarrhoea, porcine reproductive and respiratory syndrome and salmonellosis were used in this study.

The castrates had been weaned when they were 5 weeks old. They were between 10 and 11 weeks old (22-25 kg) when transferred to the experimental unit at the Department of Animal Nutrition and Management, Uppsala, Sweden. All pigs came from different litters. At the termination of the trial, the pigs weighed between 38.5 and 43.6 kg and were 14-15 weeks old.

Housing and feeding

The pigs were kept in separate pens with straw and the rooms were illuminated between 7:00 am and 7:00 pm every day. Each pen had a stone and a chain for the pigs to play with. A standard pig feed (Singel Flex, ODAL, Sweden) was offered twice daily at 7:30 am and 3:30 pm. The pigs were fed at a level of 4% of the mean live weight of the group. Water was available ad libitum. The health status of the animals was inspected at least twice daily and special attention was taken to keep the pigs washed and clean.

Surgery

The pigs were acclimatised to the new environment for 7 days. Then (11 to 12 weeks of age; 27.5-33.0 kg body weight) they were surgically fitted with a Post Valve T-Caecum (PVTC) cannula as a preparation to a feeding trial. The construction and insertion of the cannula followed the procedure previously described by *van Leeuwen et al.* (1991). Lanolin-based zinc oxide cream was used to avoid skin irritation around the PVTC-cannula, and the surgical stitches were removed after 9-11 days.

Prior to the surgery, the pigs were premedicated

by one intramuscular injection with 75 mg each of tiletamin and zolazepasam (Zoletil forte vet., Virbac Laboratories, Carros, France) and 60 mg Azaperon (StresnilTM, Jansson & Cilag pharma, Wien, Austria). Within 30 min anaesthesia was induced and maintained by inhalation of O₂ and halothane. Post surgery the pigs were intramuscularly injected once with a long acting oxytetracycline (20 mg per kg body weight; Terramycin[®] vet. Prolongatum, Pfizer, New York, USA), and they were also given an intramuscular injection of 0.3 mg buprenorphinum (Temgesic[®], Reckitt & Coleman, Hull, England) to reduce post-operative pain. Two of the pigs received one more injection of buprenorphinum on the day after surgery and 5 of the pigs received 2 more injections on the 2 days following surgery.

Sampling procedures

To document the enteric coliform flora before inserting the cannula, ileo-caecal and rectal samples were collected with a cotton swab during the surgery. To evaluate the influence of surgery and antimicrobial treatment, similar samples were collected 3, 7, 14 and 20 days post surgery.

Biochemical fingerprinting of coliforms

All samples were spread on blood agar (blood agar base No.2; LabM, Salford, England + 5% horse blood) and incubated for 24 h at 37°C. After incubation, the bacterial growth were homogenized and dispersed in broth and frozen in -20°C until analysed. Then they were spread on McConkey agar and incubated for 24 h at 37°C. Twenty-four colony forming units from each sampling site and day were scrutinised by biochemical fingerprinting. Overall 1680 isolates were analysed using the Phene Plate (PhP) system (Ph Plate, Stockholm AB, Sweden). The isolates were inoculated on PhP-RS plates (Pheneplates[®], Biosys, Stockholm, Sweden), a

system measuring the kinetics of bacterial growth in liquid medium in microtitre plates (Kühn *et al.* 1985, Möllby *et al.* 1993). Each microtitre plate contains 11 dehydrated reagents, chosen to differentiate between coliforms (Kühn *et al.* 1993). The metabolic response of each bacterial isolate to every substrate was measured at 620 nm using a microplate reader (Titertek Multiscan MCC/340, Labsystems OY, Helsinki, Finland) after 4, 7, 24 and 48 h of incubation at 37°C. The mean value of all readings was taken as the metabolic fingerprint for each isolate, and a dendrogram was constructed after pairwise comparison of biochemical fingerprints (Katouli *et al.* 1992). Isolates showing higher similarities than the established identity level (97.5%) were regarded as identical and assigned to the same biochemical phenotype (BPT). The phenotypic diversity of the coliforms was measured as Simpsons index of diversity (Hunter & Gaston 1988). The diversity is high (max value = 1) for a population comprising different BPTs and low (0) if only one BPT is present.

Statistics

The effect of sampling day on diversities for the coliform populations at each sampling site were compared by the GLM procedure of SAS version 6.12 (SAS 1998). Pig and sampling day were used as main effects in the model. Results are presented as least square means with standard errors. Students *t*-test was used when means of the coliform diversity in the ileo-caecal ostium and in rectum were compared.

Results

Health status and feed intake

The pigs remained healthy during the convalescence period and showed no signs of disease following surgery. They ate all the feed they were offered and no feed refusals were observed.

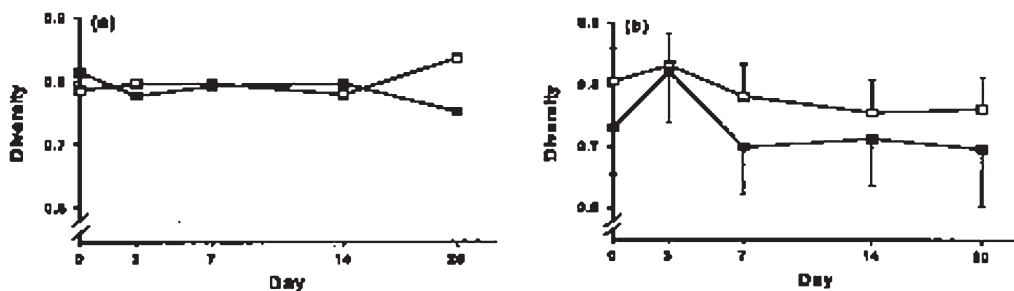


Fig. 1. Median (a) and mean values with standard error (b) of the diversity of the enteric coliform flora in the ileo-caecal ostium (open squares) and rectum (filled squares) of 7 conventional Yorkshire castrates following insertion of an ileo-caecal cannula on day 0. To minimise the risk for postoperative infections a single intramuscular injection with a long acting derivate of oxytetracycline was given in connection to surgery. At surgery the pigs were aged 12 weeks.

Metabolic fingerprinting

The diversity of the coliform populations were studied in 24 isolates per pig and sampling site each day of sampling. In total 1680 isolates were scrutinised. The diversity was high before surgery, both at the ileo-caecal ostium and in rectum (Fig. 1). Following surgery and oxytetracycline treatment, no changes in the diversity of the enteric coliform populations were observed in any of the investigated spots (Fig. 1). Further, the diversity of the enteric coliform flora was similar at these sites of sampling (the ileo-caecal ostium and the rectum). Thus, no statistically significant differences within group over time, or between groups at the different sampling occasions were observed.

Discussion

The composition of the enteric coliform populations with respect to diversity is previously shown to reflect the entire intestinal microflora well (Kühn *et al.* 1993, 1995, Katouli *et al.* 1995, 1997, Melin *et al.* 1997). In the present study the diversity of the coliform flora was not influenced by gut surgery, corresponding well to the results obtained in a recently published report on the same topic (Jacobson *et al.* 2001).

However, in that study no post surgery treatment with antibiotics was given. Treatments with antibiotics are commonly used post surgery to minimise the risk for complications by infections. However, they may well simultaneously manipulate the enteric flora, as has been shown earlier (Lüdke *et al.* 1987, Varel *et al.* 1987, Hansen *et al.* 2000). To minimise that risk, oxytetracycline was chosen for the post surgery treatment. Oxytetracycline has a specific ability to chelate with divalent metal ions (Farrington *et al.* 1991, Carson & Breslyn 1996), and an intestinal chelation reaction which inactivated the oxytetracycline excreted to the gut probably occurred. Further, the stress when administering the drug parenterally was minimised by using a single injection of a long acting derivate of the drug. Also a comparably high resistance towards the drug among coliforms (Melin *et al.* 2000) may have contributed to the stability of the enteric coliform flora during the post surgery period. Therefore, the results obtained suggest that neither the surgery itself and the somewhat altered conditions achieved at the ileo-caecal ostium, nor the par-enteral treatment with oxytetracycline influ-

enced the enteric microflora at the locations investigated.

Further, the diversity of the coliform flora in rectum matched that of the coliforms at the ileo-cecal ostium. This finding is in agreement with earlier results (Zoric *et al.* 2001), indicating that the rectal flora mirror the flora of the posterior intestine well. These authors showed that the microbial diversity of the coliforms was similar in jejunum, ileum, caecum, colon and rectum. Despite this, the coliform flora as such differed somewhat between the different segments of the intestine (Zoric *et al.* 2001). The latter observation was however not surprising because different parts of the gut have different functions in the segments investigated.

In conclusion, the results from this study indicate that the PVTC-cannulation technique offers unique possibilities to study processes in the small intestine in living pigs without affecting the enteric microflora. If parenteral antibiotic treatment is to be initiated to reduce the risk for post operative complications, oxytetracycline appears to be a good choice because of a minor influence on the enteric flora.

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Sammanfattning

Inverkan av ileo-cekal fistulering och oxytetracyklin på de ileo-cekala och rektala coliforma populationerna hos grisar.

Genom ett operativt ingrepp försågs sju 30-kilos Yorkshire-kastrater med en ileo-cekal fistel för provtagning av ingesta. Efter genomförd operation gavs grisarna en intramuskulär injektion med ett långtidsverkande derivat av oxytetracyklin. Hos samtliga grisar togs ileo-cekala och rektala prov i samband med operationen samt 3, 7, 14 och 20 dagar efter ingreppet. Proverna användes för studier av eventuella förändringar av den coliforma ileo-cekala och rektala mikrofloran och totalt analyserades 1680 isolat. Studien visade att den mikrobiella diversiteten initialt var hög både vid det ileo-cekala ostiet och i rektum. Ingen förändring kunde observeras i diversiteten hos den coliforma floran efter operationen och behandlingen med oxytetracyklin. En trolig förklaring till att den coliforma tarmfloran inte påverkades av behandlingen med oxytetracyklin kan vara en inaktivering av den substans som utsöndrats till tarmen genom bindning till tvåvärdade joner i ingestan. Vår slutsats är att den fistuleringsteknik som studerats endast marginellt kan förväntas påverka den coliforma tarmfloran hos grisarna och därför kan anses väl lämpad för in vivo studier av såväl foderutnyttjande som andra processer i tunntarmen.

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